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**13. Abstract** ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

There is abundant evidence that increased expression of growth factors and increased activity growth factor receptors, particularly those of the fibroblast growth factor (FGF) and epidermal growth factor (EGF) families, play an important role in human prostate cancer. The sprouty gene was originally identified as a negative regulator of FGF and EGF receptor signaling in Drosophila. Based on our preliminary data, sprouty-1 is the major human sprouty homologue expressed in human prostate and it is significantly downregulated in approximately 70% of extensive, clinically localized prostate cancers. Loss of this negative regulator of growth factor signaling may enhance tumor aggressiveness and be correlated with clinical, pathological and biological parameters of aggressive clinical behavior in human prostate cancer. We have now shown that expression of sprouty-1 in human prostate cancer cells, either by stable transfection or by expression via replication deficient adenovirus, markedly inhibits proliferation of prostate cancer cell lines. Future work, using the reagents during this initial funding period or currently being generated, will allow us to comprehensively evaluate the expression of sprouty-1 in human prostate cancer and determine its effect on the biological behavior of prostate cancer cells both *in vitro* and *in vivo*.

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## INTRODUCTION

There is abundant evidence that increased expression of growth factors and increased activity growth factor receptors, particularly those of the fibroblast growth factor (FGF) and epidermal growth factor (EGF) families, play an important role in human prostate cancer. Recently, a new family of regulators of FGF and EGF activity has been identified. The sprouty gene was originally identified as a negative regulator of FGF and EGF receptor signaling in Drosophila and it has been proposed that it may carry out these two functions by interfering with the RAS signaling pathway. Three human homologues have been identified, but their role in the human prostate and prostate cancer is not well understood. Based on our own preliminary data and analysis of the Cancer Genome Anatomy and Unigene databases, sprouty-1 is the major human sprouty homologue expressed in human prostate and it is significantly downregulated in approximately 70% of extensive, clinically localized prostate cancers. Loss of this negative regulation may lead to unrestrained signal transduction by FGF and EGF family members, which could result in increased proliferation and may enhance tumor aggressiveness and be correlated with clinical, pathological and biological parameters in human prostate cancer.

## BODY

As outlined in our Statement of Work, we have sought to accomplish five tasks in our initial funding period. Most of these tasks are prerequisites to carrying out the definitive biological experiments and molecular analysis in years 2 and 3 of this proposal. We have accomplished or made substantial progress on all of these tasks.

*Task 1: Clone full length human sprouty-1 cDNA (Months 1-3)*

We have cloned a full-length sprouty-1 cDNA and this has been sub-cloned into the pCEP4 expression vector as well as pAVS6 for production of adenovirus.

*Task 2: Construct adenovirus expressing sprouty-1 gene (Months 3-12)*

We have generated a recombinant sprouty-1 adenovirus and carried out large scale preparations of this virus. We have confirmed that this adenovirus expresses sprouty-1 protein by Western blotting with anti-sprouty-1 antibody using extracts of LNCaP cells infected with either sprouty-1 adenovirus or control green-fluorescent protein (GFP) adenovirus. Preliminary biological experiments reveal that this sprouty-1 adenovirus markedly inhibits colony formation (Fig 1) and net proliferation (Fig 2) in LNCaP and DU145 cells.

*Task 3: Develop prostate cancer cell lines with high sprouty-1 expression (Months 3-12)*

As described above we have we have cloned the full length sprouty-1 cDNA into the pCEP4 expression vector. We have performed stable transfection of the LNCaP cell line, isolated stable transfectants and evaluated for sprouty-1 expression by quantitative RT-PCR. Analysis of the growth rate of these cell lines reveals that there is inhibition of growth relative to parental LNCaP cells (Fig 3). It should be noted that it was difficult to establish cell lines expressing sprouty-1 due to low numbers of colonies following transfection. Thus it is likely that the clones obtained are those with relatively low sprouty-1 expression, with higher expressing clones lost due to inhibition of proliferation by sprouty-1. Similar experiments are underway with PC3 prostate cancer cells.

*Task 4: Clone sprouty-1 genomic locus, identify intron-exon structure and identify promoter (Months 1-12)*

We have cloned the sprouty-1 gene as a single BAC clone. We have identified the intron-exon boundaries and developed PCR primers for mutation analysis by analysis of human genome project database. Fortunately, the sprouty-1 gene consists of only two exons, facilitating mutation analysis (Fig 4). We have analyzed 30 human prostate cancer DNAs and have found no evidence of mutation, consistent with our initial hypothesis that alteration of mRNA expression is the primary mode by which sprouty-1 activity is decreased in prostate cancer. We have also determined the sequence of promoter region from database. We have verified its activity as promoter by construction of plasmid with 850 bp of the putative promoter driving a Luciferase reporter gene which was then transiently transfected into NIH3T3 cells. This construct expresses Luciferase robustly in NIH3T3 cells and transcription is upregulated by exogenous FGF2 (Fig 5). Finally we have isolated a probe for methylation analysis.

*Task 5: Raise and characterize rabbit polyclonal anti-sprouty-1 antisera (Months 1-12)*

We have recently obtained a commercially available anti-sprouty-1 antibody which we have used in testing our adenovirus construct. We are currently analyzing this antibody for its suitability for use in immunohistochemical analysis of paraffin embedded prostate cancers. We are also actively immunizing rabbits with two different sprouty-1 peptides and should have antisera to test shortly. In addition, we have constructed a His-tagged sprouty-1 fusion protein to use as an antigen if our peptides do not elicit high titer antibodies.

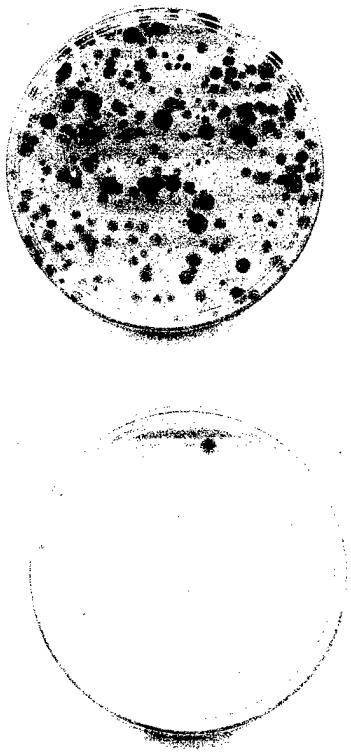


Figure 1. Colony formation after treatment of DU145 cells with sprouty-1 adenovirus (lower) compared to cells treated with GFP adenovirus (upper). Essentially identical results were obtained using LNCaP cells (data not shown).

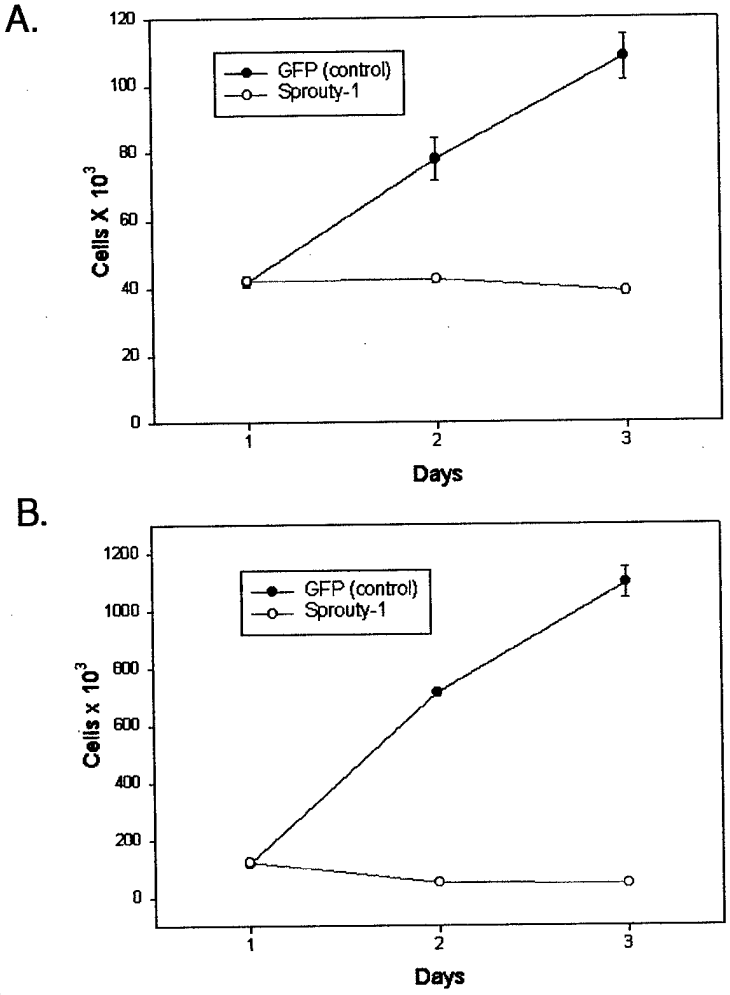



Figure 2. Proliferation of LNCaP (A) and DU145 (B) prostate cancer cells treatment with replication deficient adenovirus expressing sprouty-1(red) or GFP expressing adenovirus(black). Standard errors of mean of triplicate determinations are shown. The differences in cell number at days 2 and 3 were statistically significant in both cases (P< 0.01, t test)

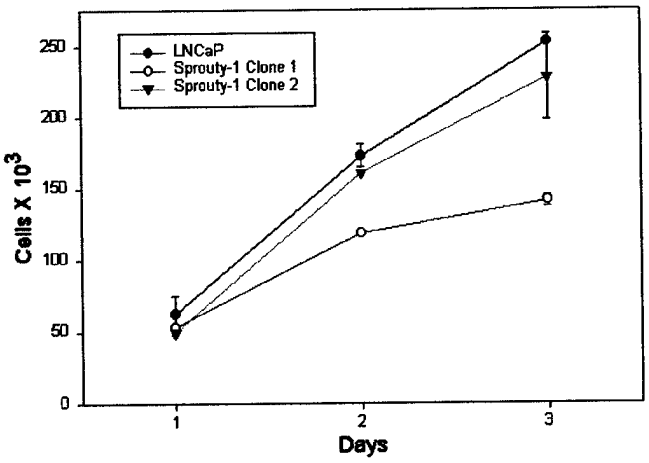
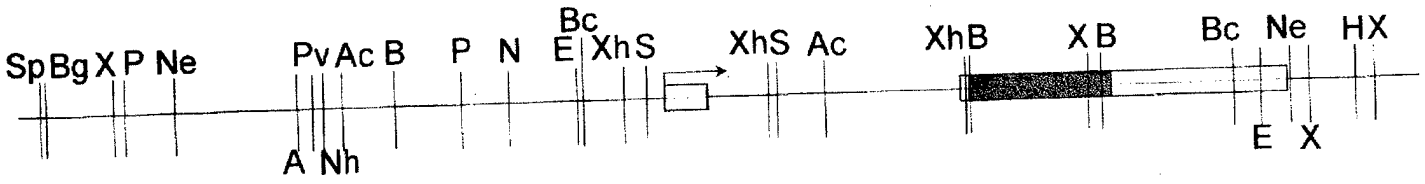



Figure 3. Proliferation of LNCaP clones expressing sprouty-1 compared to control cells. The difference in proliferation of clone 2 relative to control was statstically significant at days 2 and 3 ($p<0.01$, t test). Standard errors of the mean of triplicate determinations are shown.

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|---|---|---|---|
| P=PstI | Sp=SpeI | E=EcoRI | Pv=PvuI |
| Bc=BclI | Bg=BglII | Ne=NdeI | Nh=NheI |
| Xh=XhoI | X=XbaI | S=SacI | Ac=AclI |
| H=HindIII | N=NotI | A=AgeI | B=BamHI |

Figure 4. Overview of sprouty-1 genomic locus. Exons are indicated as boxed areas with translated sequence in green. Restriction sites are indicated.

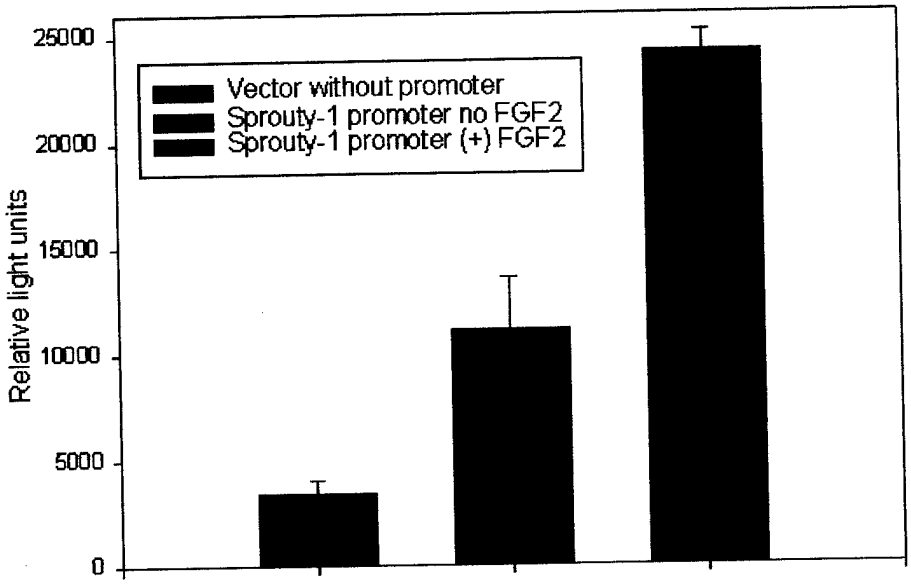


Figure 5. Luciferase activity 24 hrs following transfection of Luciferase vector without promoter fragment or containing promoter fragment in the presence or absence of 100 ng/ml FGF2. Extract protein used for Luciferase assay was normalized using beta-galactosidase to control for variation in transfection efficiency. Mean and range of duplicate determinations are indicated.

## KEY RESEARCH ACCOMPLISHMENTS

* Cloned full-length sprouty-1 cDNA and subcloned into mammalian expression vector

* Created replication deficient adenovirus expressing sprouty-1 and demonstrated that it markedly inhibits proliferation and colony formation by prostate cancer cells

* Established stably transfected LNCaP cell lines that express sprouty-1 and shown that these cell lines have decreased proliferation relative to controls.

* Cloned sprouty-1 genomic locus and established intron-exon structure of sprouty-1 gene

* Performed mutation analysis of the sprouty-1 gene in 30 human prostate cancers.

* Cloned an 850 bp promoter fragment of the sprouty-1 gene and demonstrated that it will act as an FGF regulated promoter in NIH3T3 cells.

* Identified potentially immunogenic sprouty-1 peptides and started production of anti-sprouty-1 antisera.

* Constructed His-tagged sprouty-1 fusion protein.

## REPORTABLE OUTCOMES

* Created sprouty-1 mammalian expression vector.

* Created replication-deficient adenovirus expressing sprouty-1.

* Established prostate cancer cell lines expressing sprouty-1.

## CONCLUSION

Sprouty-1 expression is downregulated in the majority of human prostate cancer that we have evaluated to date. The work described above reveals that sprouty-1 expression profoundly inhibits proliferation of prostate cancer cells *in vitro*. Future work using the reagents described above or currently being generated will allow us to comprehensively evaluate the expression of sprouty-1 in human prostate cancer and determine the effect of sprouty-1 expression on the biological behavior of prostate cancer cells both *in vitro* and *in vivo*.